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Eremiomyces magnisporus (*Pezizales*), a new species from central Spain

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Abstract — A new species of *Eremiomyces* is described from central Spain. *Eremiomyces magnisporus* is proposed to accommodate a single collection found in July 2010 in a marl-gypsicolous soil at Mount Gurugú, Alcalá de Henares (Spain). The habitat and macro-/micromorphology of the fresh material are described. Molecular analyses of ITS and nLSU DNA support the new species as closely related to but distinct from *Eremiomyces echinulatus* from the Kalahari desert.

Key words — Kalahari truffles, Mediterranean, hypogeous fungi

Introduction

The genus *Eremiomyces* Trappe & Kagan-Zur must be considered as a member of the *Pezizaceae* Dumort. (*Pezizales* J. Schröt.) since it was proposed by Ferdman et al. (2005) to accommodate the molecularly deviant species *Choiromyces echinulatus* Trappe & Marasas [≡ *E. echinulatus* (Trappe & Marasas) Trappe & Kagan-Zur]. *Eremiomyces echinulatus* is a truffle of the African Kalahari, collected in South Africa, Botswana, and Namibia (Trappe et al. 2008, 2010) and, until now, the only known species in this genus.

In the present work, a second *Eremiomyces* species is described from the steppe-like semi-arid hills around Alcalá de Henares, central Spain. These low hills present a marl-gypsum soil covered by introduced *Pinus halepensis* as well as indigenous xerophilous shrubs such as *Macrochloa tenacissima*, a Mediterranean poaceous plant with an Iberian and northern African distribution (Figs. 1–2). Other fungi previously collected in this region include *Battarrea phalloides* (Dicks.) Pers., *Dictyocephalos attenuatus* (Peck) Long & Plunkett, *Galeropsis desertorum* var. *bispora* (Vassilkov) G. Moreno et al., *Gastrosporium simplex* Mattir., *Tulostoma* spp., and *Phaeomyces ibericus* (G. Moreno & Esteve-Rav.) E. Horak.

Figs. 1. Habitat under *Pinus halepensis* with *Macrochloa tenacissima*. 2. Sample collection site.

Materials & methods

Morphology

The type material is preserved in the herbarium of the Dept. of Plant Biology, University of Alcalá de Madrid (AH). Microscopical observations were recorded from tissues mounted in KOH 5%, lactophenol cotton blue, and congo red. Spore measurements do not include superficial structures. Scanning electron microscopy (SEM) images were taken at the University of Alcalá de Henares, using a Zeiss DSM-950 instrument.

DNA extraction, PCR amplification, and DNA sequencing

DNA extraction, PCR amplification, and amplicon purification from type collection AH 38981 was performed as described previously (Alvarado et al. 2010). PCR primers ITS1F and ITS4 (Gardes & Bruns 1993), and ITS4 (White et al. 1990) for the ITS region, and LR1 and LR7 (van Tuinen et al. 1998, Vilgalys & Hester 1990) for the nLSU rDNA region were employed for PCR amplification and sequencing purposes. Sequences were visually inspected for reading errors in MEGA4 (Tamura et al. 2007).

Sequence alignment and phylogenetic analysis

The obtained sequences were aligned with the closest matches from BLAST searches in the public databases. ITS and nLSU sequences came mainly from Læssøe & Hansen (2007) and Perry et al. (2007). Sequences were aligned in MEGA4.0 software using its ClustalW application. The final alignment was assembled manually. Neighbour-joining phylogenetic inference was performed in MEGA4 (pairwise deletion of gaps, 2000 bootstrap replicates). The aligned loci were loaded in PAUP* 4.0b10 (Swofford 2001) and a maximum parsimony phylogenetic tree reconstruction was performed (2000 bootstrap replicates, TBR swapping algorithm, 50 sequence additions per replicate, MULTrees not in effect). Aligned loci were also subjected to MrModeltest 2.3 (Nylander 2004) in PAUP* 4.0b10 to search for the evolutionary models that best fit the data. These models were simulated during the analysis of each locus in MrBayes 3.1 (Ronquist & Huelsenbeck 2003). A Bayesian maximum likelihood (BML) analysis using 4 metropolis-coupled Monte Carlo Markov Chains (MCMC) was performed setting the temperature of the chains to 0.2 and sampling once each 100 generations. The analysis was run until convergence parameters were met in two simultaneous runs, after about 2M generations. A full search for the highest scoring ML trees was also performed in RAxML (Stamatakis 2006) using the standard search algorithm, a thousand bootstrap replications, and allowing an independent evolutionary model for each locus.

Molecular results

The final alignment of the ITS region included 327 of 658 variable sites, 288 of which were also parsimony-informative. In turn, nLSU alignment included 114 parsimony-informative among 151 variable sites from a total 592 bases. Both ITS and nLSU data were best described by the evolutionary model GTR+G+I.

Taxonomy

Eremiomyces magnisporus G. Moreno, P. Alvarado, Manjón & Sanz **sp. nov.**

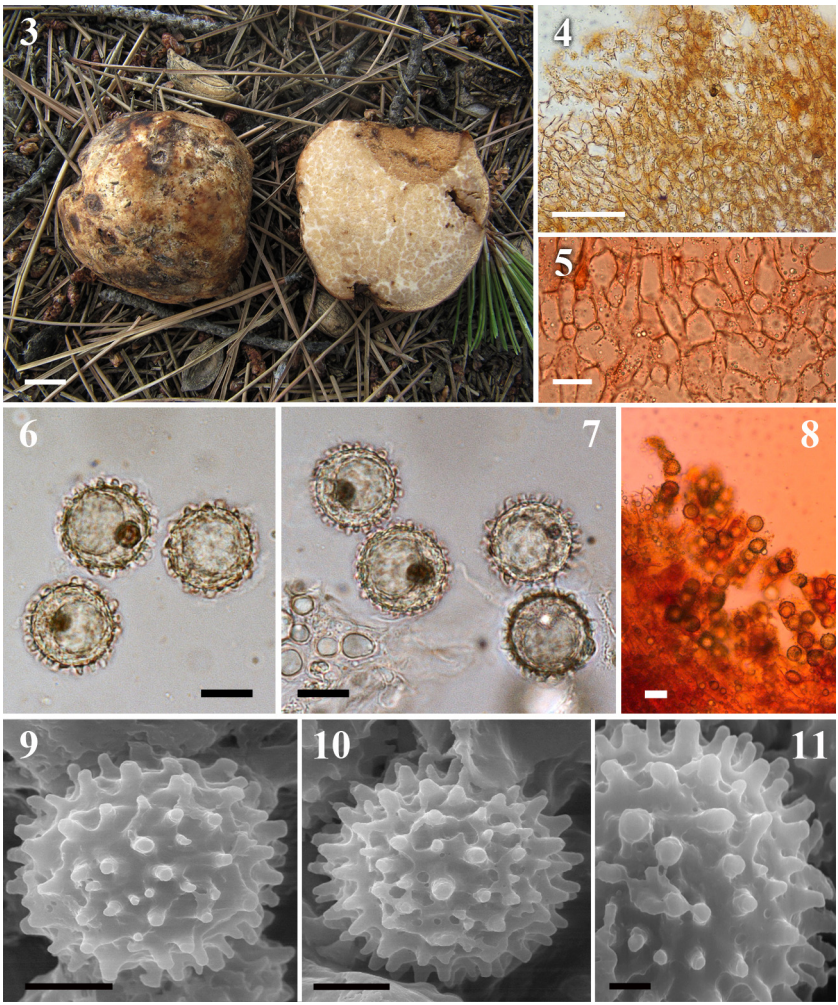
MycoBank MB561665 Figs. 3–11

Ascomata subglobosa, 5 cm longa, peridio pallide brunneo passim atrorufo. Colores iuvenum speciminum permanent post siccationem. Peridium 0.1–0.3 mm crassum, angularis structura, magnis cellulis 25–45 × 5–20 µm composita, praeditum. Gleba griseobrunnea vel griseostraminea numerosis albidis venis incomposite distributis, formantibus varium reticulum. Sporae 14–17 µm sine ornamentis, globosae, hyalinis vel pallide luteolis vacuolis praeditae. Sporarum ornamenta ex obtusis bacillis vel conis dense distributis, in mente revocantibus ornamenta Terfeziae arenariae, *sed perspicue breviora (1.5–2 µm longa, 1 µm lata) et magis dense disposita, efformata. Asci cylindrici difficiles inventu aetate provecta speciminis, videntur una serie dispositi. Paraphyses haud observatae. Odor fortis revocans caseum domesticum*.

Type — Spain, Madrid, Alcalá de Henares, Mount San Juan del Viso, 750 m a.s.l., 10.VII.2010, leg. M.A. Sanz (**Holotype** AH 38981). [GenBank JN032128 (ITS), JN032129 (28S nLSU)].

Etymology — Latin, *magni-* (big) + *-sporus* (spores), referring to the larger size relative to those in *E. echinulatus*.

Ascoma subglobose 5 cm diam., without an obvious attachment point to the substrate, peridium smooth, pale brown with reddish to reddish-black areas where damaged, fresh colours retained after drying. Peridium 0.1–0.3 mm thick, with an angular structure formed by entangled cylindrical hyphae; cells 25–45 × 5–20 µm, becoming more globose toward the interior; cell walls ≤ 1.5 µm, hyaline to slightly yellowish; outermost cell layer 10–40 µm thick, red-brownish vacuolar pigments present; external layer of amorphous debris ≤ 25 µm thick. Gleba beige to dun straw with abundant 0.05–0.4 mm broad whitish veins irregularly distributed forming a variable reticulated mesh with multiple attachment points to the peridium, colour appearing darker where bitten by animals; once dried, small [0.5–1.5 mm diam.] (sub)globose or subglobose spore-containing pockets protrude. Ascospores globose, 14–17 µm (average 15.875, SD 0.545) without ornamentation, hyaline to slightly yellowish, vacuolate; ornamented by densely arranged 1.5–2 × 1 µm blunt-edged rods and cones reminiscent of *Terfezia arenaria* (Moris) Trappe, but clearly shorter and more densely arranged. Asci and paraphyses could not be found due to the advanced maturity of the sample, but spores remained linearly arranged, commonly in groups of 4(–5) spores. Odour strong, similar to cottage cheese.



Figs 3–11. *Eremiomyces magnisporus* (Holotype: AH 38981). 3. Ascoma peridium and gleba. 4. Peridium. 5. Detail of angular peridial cells. 6–7. Spores under LM. 8. Linearly arranged spores. 9–10. Spores under SEM. 11. Detail of spore ornamentation under SEM.
Scale bars: 3 = 1 cm, 4 = 100 m, 5 = 25 µm, 6–7 = 10 µm, 8 = 20 µm, 9–10 = 5 µm, 11 = 2 µm.

Ecology & distribution. *Eremiomyces magnisporus* is a rare species fruiting in steppe-like areas of the marl-gypsum hills surrounding Alcalá de Henares (central Spain).

Comments. A single ascoma was located by the truffle-hunting dog of Miguel Ángel Sanz under *Macrochloa tenacissima* and *Pinus halepensis* in July 2010.

Figure 2 shows the exact point of collection under *M. tenacissima*, without any sign of the typical 'burn' associated with other hypogeous fungi. It cannot be concluded which plant species was host of this presumably mycorrhizal fungus. Kalahari truffles have been said to fruit under poaceous species: *Enneapogon cenchroides* (Licht.) C.E. Hubb., *Eragrostis rigidior* Pilg., and *Stipagrostis uniplumis* (Licht.) De Winter (Trappe et al. 2008). Interestingly, *E. echinulatus* was collected in June during the austral winter (Trappe et al. 2010), while *E. magnisporus* appeared in northern hemisphere during the summer.

Eremiomyces magnisporus is characterized by its pale brown peridium with reddish areas that do not blacken after drying, large-sized spores (14–17 µm without ornamentation), and a spore ornamentation formed by very densely arranged blunt-edged hyaline to pale yellow cones. *Eremiomyces echinulatus* is distinguished by a peridium that turns black after drying, dark brown glebal veins, and smaller spores (10–14 µm without ornamentation).

Discussion

Phylogenetic similarities between South African and Mediterranean fungi have been reported before. The recently proposed inocybeoid genus *Tubariomyces* Esteve-Rav. & Matheny is represented in both Spain and Italy and Zambia (Alvarado et al. 2010). *Tubariomyces* species apparently associate with *Cistaceae* Juss. in the Mediterranean and with *Phyllanthaceae* Martinov and *Fabaceae* Lindl. in Zambia.

Macrochloa tenacissima, the putative host plant of *E. magnisporus*, is commonly found in semiarid basic soils of the Mediterranean basin in Turkey, Italy, Spain, Morocco, Algeria, Tunisia, and Libya. In the western Mediterranean, it reaches central Spain and several more scattered spots of northeastern Spain, yet it is not found beyond the high plateaus preceding the Sahara desert to the south and southwest of Morocco. Up to now, *M. tenacissima* has been regarded as an exclusively endomycorrhizal plant, hosting glomalean species such as *Glomus aggregatum* N.C. Schenck & G.S. Sm. and *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler (Roldán-Fajardo 1994). Interestingly, the hypogeous fungus *Gastrosporium simplex* has also been found in association with poaceous species of the *Arrhenathero-Stipetum tenacissimae* association in the hills around Alcalá de Henares (Moreno et al. 1991) as well as with *Festuca ovina* L. (Montecchi & Sarasini 2000).

Of the poaceous genera indicated by Trappe et al. (2010) as the most probable hosts of the Kalahari truffles, those most closely related to *Macrochloa* are *Stipagrostis* Nees, *Eragrostis* Wolf and *Enneapogon* Desv. ex P. Beauv. Although widely distributed, *Eragrostis* is considered typical of subtropical and xerophilous habitats and is common in Spain. Likewise, *Enneapogon* has a split Australian–African distribution, with only *E. desvauxii* P. Beauv. being

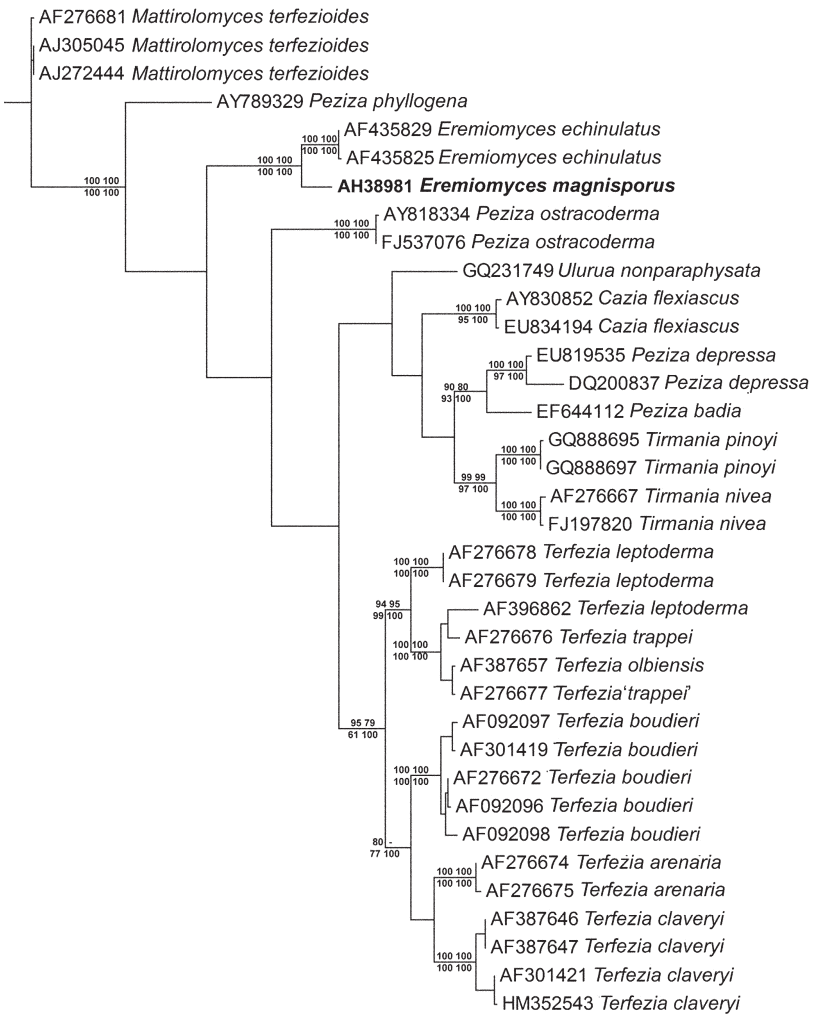


Fig. 12. *Eremiomyces magnisporus* and its closest relatives in the *Pezizaceae*. Highest scoring ITS tree (maximum parsimony, PAUP*). Only nodes supported by three or more of the inference methods are annotated. Values represent (from upper left to lower right): MEGA4 neighbour-joining, PAUP maximum parsimony, RAxML bootstrap proportions, and MrBayes posterior probabilities.

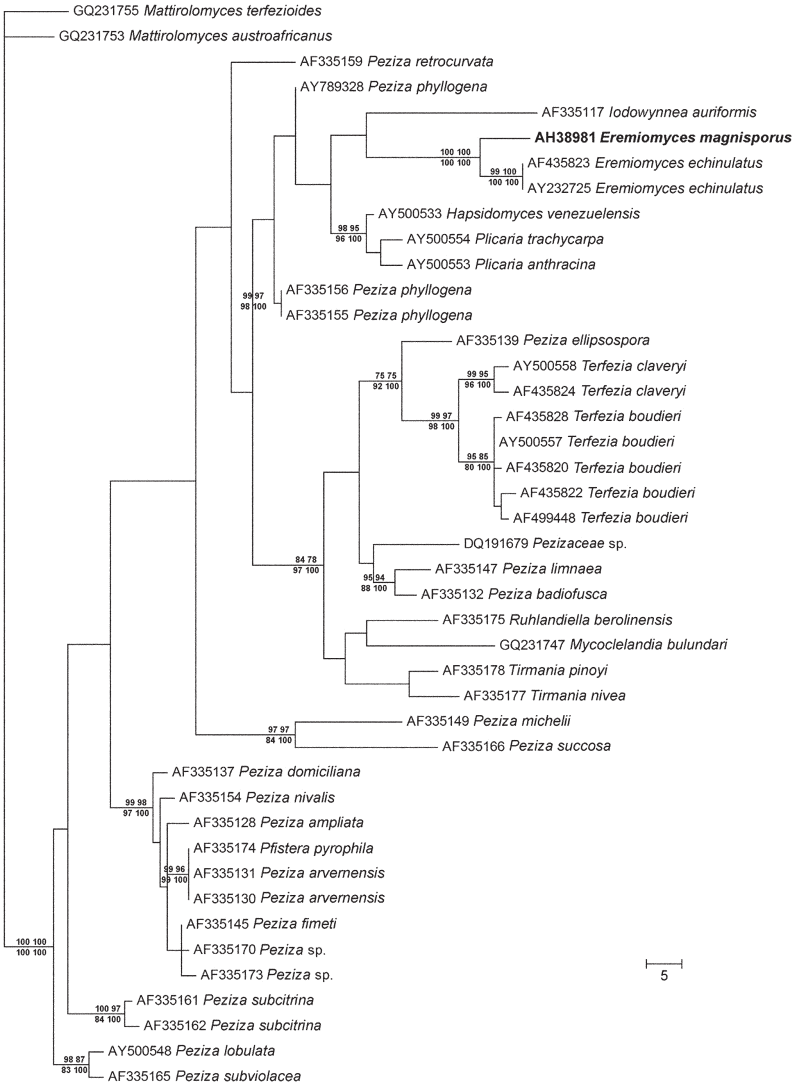


Fig. 13. *Eremiomyces magnisporus* and its closest relatives in the *Pezizaceae*. Highest scoring nLSU rDNA tree (maximum parsimony, PAUP*). Only nodes supported by three or more of the inference methods are annotated. Values represent (from upper left to lower right): MEGA4 neighbour-joining, PAUP maximum parsimony, RAxML bootstrap proportions, and MrBayes posterior probabilities.

widespread. Most African *Enneapogon* species range from south to northeastern Africa, often reaching Arabia and India (Peterson et al. 2010). Only *E. persicus* Boiss. reaches western Mediterranean to southeastern Spain. In turn, *Stipagrostis* is found from southern Africa to Middle East and northwestern India, reaching the western Mediterranean up to Morocco (Le Houérou 2001). It would be interesting to search for this and other hypogeous genera in the distribution areas of the poaceous species mentioned above. More research on *Eremiomyces* and its unexpected plant hosts is clearly needed to shed light on its disjunct geographical distribution

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